

# Greenhouse Investigations on the Effect of Guava on Infestations of Asian Citrus Psyllid in Grapefruit

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Reports from Vietnam indicate interplanting guava with citrus reduces infestations of Asian citrus psyllid (ACP) (*Diaphorina citri*) in citrus. We therefore conducted cage studies in a greenhouse to assess the effect of different guava cultivars on adult ACP mortality and settling behavior on citrus (young potted grapefruit). The effects of cotton and tomato were also evaluated in some tests as non-citrus, neutral host species. Survival of adult ACP confined to potted guava in no-choice situations was reduced as compared to survival on potted grapefruit. However, adult survival was also reduced when they were confined to potted cotton or tomato. Adult ACP released into cages containing only citrus generally moved faster to citrus than when either 'White' guava or cotton was present. Greater numbers of adults were consistently observed on citrus over time in cages with only citrus as compared to in cages with citrus in the presence of guava or cotton. This may have been due to differences in the total plant surface area in cages with citrus alone compared to citrus caged with another plant. Mortality rates of adults were increased in cages containing both citrus and guava in one of two studies. While significant reductions in infestations of adults on young grapefruit sometimes occurred in cages containing both citrus and guava in the greenhouse, the reductions were not enough to verify the Vietnamese guava effect.

The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), is an important pest of citrus in Florida, primarily because it is a vector of "*Candidatus* Liberibacter asiaticus" (Halbert and Manjunath 2004). This and other species of "*Ca. Liberibacter*" are phloem-limited, non-culturable bacteria responsible for citrus greening disease (huanglongbing) (Halbert and Manjunath, 2004; Hung et al., 2004). Citrus greening is considered to be one of the world's most serious diseases of citrus (Bové, 2006). Citrus trees infected by this devastating disease may only live 5 to 8 years, during which time they produce misshapen, inedible fruit (Bové, 2006; Gottwald et al., 2007). ACP was first found in Florida during June 1998 (Tsai and Lui, 2000) and is now established throughout the state's citrus-growing regions (Michaud, 2004). Citrus greening was found in Florida during late Aug. 2005 (Gottwald et al., 2007) and was subsequently shown to be widespread, especially in southern areas of the state.

Recent reports from Vietnam indicate that infestations of

ACP and, consequently, incidences of citrus greening disease in citrus are greatly reduced when citrus is interplanted with guava, *Psidium guajava* L. (plant family Myrtaceae) (Beattie et al., 2006). These observations were made in high density plantings of citrus that were intercropped with guava at a one-to-one tree ratio [2.5-m (8.2 ft) row spacing, 2.5-m (8.2 ft) tree spacing along rows]. It is speculated that guava volatiles or phytotoxins might be responsible for reducing infestations of the psyllid on citrus. Putative guava volatiles may interfere with the psyllid's ability to locate and infest citrus grown next to guava, or they might repel psyllids away from citrus. Putative guava toxins might negatively affect the biology of the psyllid, interfering with psyllid reproduction in citrus.

The reports from Vietnam prompted greenhouse investigations in Florida. Three experiments were conducted to assess survival of adult psyllids confined to citrus, guava (cultivars available in Florida), and other plants in no-choice situations. Two experiments were conducted to assess settling behavior and survival of adult psyllids in cages containing a young citrus plant by itself, in cages containing both young citrus and guava plants, and in cages containing both young citrus and cotton plants.

## Materials and Methods

The insects for the studies were obtained from a colony established during early 2000 at the USDA-ARS U.S. Horticultural Research Laboratory, Fort Pierce, FL. Originally collected from

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citrus, the psyllids have since been continuously reared in Plexiglas (0.6 × 0.6 × 0.6 m) (2 × 2 × 2 ft) or BugDorm-2 cages (60 × 60 × 60 cm) (24 × 24 × 24 inches) (MegaView Science Education Services Co., Ltd., Taichung, Taiwan) containing potted orange jasmine, *Murraya paniculata* (L.) Jack (plant family Rutaceae). The colony is maintained by adding new *M. paniculata* plants on a bi-weekly schedule using procedures similar to those described by Skelley and Hoy (2004) with no introduction of wild psyllids.

#### Adult survival in no-choice host plant experiments

**EXPERIMENT 1.** Adults were caged in a no-choice situation on each of the following types of guava (each cultivar represented by two plants) in a greenhouse on 5 Mar. 2007: 'White' seedless guava, 'Thai' white guava, 'Ruby Supreme' guava, 'Barbie Pink' guava, 'Pink Oval' guava, and 'Duncan' white grapefruit (*Citrus paradisi* Macf., plant family Rutaceae). All of the guava plants were 80 cm (31 inches) or taller except "Pink Oval" guava, of which both plants were seedlings 8 to 13 cm (3 to 5 inches) tall. The taller guava plants were growing in 11.4-L (3 gal) pots and the small 'Pink Oval' guava plants were growing in 0.5-L (1 pt) pots. None of the guava plants had any flowers or fruit except the 'White' seedless guava plants, each of which had a few developing blooms. The grapefruit plants were seedlings 14 cm (6 inches) tall growing in 0.5-L (1 pt) pots. For the guava plants that were 80 cm (31 inches) or taller, one branch from each was selected and a 3.8L (1 gal) clear plastic container (7F18 Screw-Lid Canister, Rubbermaid, Fairlawn, OH) measuring 14.5 cm (5.7 inches) in diameter and 24 cm (9.4 inches) tall was placed over the end of the branch to house adult psyllids. We cut a narrow slit in the container's lid [11 cm (4.3 inches) in diameter, the slit extending about 6.4 cm (2.5 inches) from the edge of lid through the center of the lid], which enabled us to slip the stem of a branch into the slit of the lid and to screw the lid into place. To counter the weight of the containers, a string harness suspended from above was tied around each container. The containers had been ventilated prior to the study by cutting a 10 × 10 cm (4 × 4 inch) opening on one side of the container and gluing a screen over the opening to prevent psyllids from escaping. Each caged branch was approximately 20 cm (8 inches) in length and generally consisted of 3 or 4 mature tough leaves, 3 or 4 fully expanded new leaves, and 2 or 3 young developing leaves. Five male and five female adults (age not recorded) were placed into the container, and the slit was sealed shut using a piece of foam rubber stuffed into the slit and around the stem of the branch. The small grapefruit and 'Pink Oval' guava plants were housed as whole potted plants in the 3.8-L (1 gal) plastic containers (7F18 Screw-Lid Canisters fitted with screened lids for ventilation). Five male and five female adults (age not recorded) were placed into the container and the lid was attached. Prior to the study, Plaster of Paris was used to cover the soil of the small potted plants to expedite finding dead adults. For all infested plants, dead adults were tabulated each day except on weekends until the end of the experiment on 16 Mar. 2007.

**EXPERIMENT 2.** Procedures of this experiment were the same as those of the first adult survival experiment with respect to how psyllids were caged on plants and how many psyllids were introduced onto each plant. The exact same test plants were used except young cotton (*Gossypium hirsutum* L.) ('Tamcot 22') (plant family Malvaceae) plants growing in 0.5-L (1 pt) pots were substituted for the young 'Pink Oval' plants. With respect to the plants that had been used in the first experiment, these were maintained in the greenhouse during the 6-week interval between the end of

experiment 1 and start of experiment 2. The adult psyllids were 1 to 2 weeks old at the beginning of the test. The test was initiated 1 May 2007 and terminated 14 May 2007.

**EXPERIMENT 3.** Procedures of this experiment were the same as those of the first adult survival experiment with respect to how psyllids were caged on plants and how many psyllids were introduced onto each plant, and the same test plants were used. An exception was that young tomato (*Solanum lycopersicum* L.) (Florida dwarf 'Lanai') (plant family Solanaceae) plants growing in 0.5-L (1 pt) pots were substituted for the young 'Pink Oval' plants. With respect to the plants that were used in the second experiment, these were maintained in the greenhouse during the eight wk interval between the end of experiment 2 and start of experiment 3. The adult psyllids were about 25 d old at the beginning of the test. The test was initiated 9 July 2007 and terminated 20 July 2007.

#### Adult settling behavior and survival in choice situations

**EXPERIMENT 1.** Forty adult psyllids (20 of each sex) were released into BugDorm-2 cages (described earlier) containing either a young citrus tree ('Duncan' grapefruit) by itself (treatment 1) or a young citrus tree with a young 'White' guava tree (treatment 2) (10 replications). The young citrus trees were growing in 3.0-L (0.8 gal) pots and the guava were in 6.0-L (1.6 gal) pots. Each of the 10 cages containing a citrus plant was paired with one of 10 cages containing both citrus and guava, and each pair was randomly placed together in a greenhouse [cages 1 to 2 m (3–7 ft) apart]. Psyllids were released into the cages on 29 Mar. 2007 when they were approximately 16 d old. The number of adult ACP on each citrus tree in each cage was counted at 1.5, 3, 5, 7, 8 and 24 h after they were released into the cages and thereafter daily each morning (except on weekends) for 17 days. Data were collected daily (except weekends) on the number of dead adults in each cage. Numbers of eggs and nymphs on each citrus plant were counted on days 7, 14, and 18 of the study by excising all flush shoots from each citrus tree leaving no immatures in the cage. The total numbers of dead and live adults in each cage were counted on the last day of the experiment, 13 Apr. 2007. Data on numbers of adult psyllids settled on citrus and numbers found dead each day were plotted over time to visually compare settling behavior and survival of adults in cages with citrus alone to those in cages with both citrus and guava, and the data were compared using paired *t*-tests ( $\alpha = 0.05$ ) (PROC TTEST, SAS Institute, 2002). Mortality of adults over time in cages with citrus alone and citrus with guava was assessed using simple linear regression (PROC GLM, SAS Institute 2002).

**EXPERIMENT 2.** This experiment was the same as the first experiment with respect to assessing psyllid settling behavior and survival in BugDorm-2 cages containing citrus alone (treatment 1) and in cages with both citrus with guava (treatment 2). The same size plants were used, and the same number of male and female psyllids was introduced into each cage. However, in addition to cages with citrus alone and cages with both citrus and guava, cages with citrus and cotton (treatment 3) were included for comparison purposes. The cotton was growing in 3.0-L (0.8 gal) pots. Another difference in experiment 2 was that data on the sex of psyllids found dead each day during the study were recorded. The test was arranged in a randomized block design (blocked on location in the greenhouse) with 10 replications. Adult psyllids introduced into the cages were approximately 10 d old. The study was initiated on 15 Aug. 2007 and terminated 4 Sept. 2007. Numbers of eggs and nymphs on each citrus plant were counted



on days 7, 10, and 20 of the study following the same procedures as in the first experiment. For each observation date, numbers of adults settled on citrus and numbers of eggs and nymphs on citrus in cages with citrus alone (treatment 1) were compared to numbers on citrus in cages with citrus and guava (treatment 2), and to numbers in cages with citrus and cotton (treatment 3), using analysis of variance for the randomized complete block design (PROC ANOVA, SAS Institute, 2002), and mean comparisons were made using the Ryan-Einot-Gabriel-Welsch test. Mortality of adults over time in cages with citrus alone, citrus with guava, and citrus with cotton was assessed using linear regression (PROC GLM, SAS Institute, 2002).

## Results and Discussion

### Adult survival in no-choice host plant experiments

Greater than 50% mortality of adult psyllids occurred in the first experiment within less than 5 d when they were caged on each of the five different types of guava (Fig. 1A). All adults caged on 'White' or 'Ruby Supreme' guava were dead by 7 d. Greater than 90% of adults caged on 'Duncan' grapefruit survived during the 11 d study. In the second experiment, all adults caged on 'Duncan' grapefruit survived over the 13-d study (Fig. 1B). Similar to the first experiment, greater than 50% mortality of adult psyllids occurred within less than 5 d when they were caged on each of the four different types of guava. However, survival rates on cotton were similar to survival rates on guava. In the third experiment, all adults caged on 'Duncan' grapefruit survived over the 11 d study (Fig. 1C). Greater than 50% mortality of adult psyllids occurred within less than 3 d when they were caged on each of the four guava cultivars. Survival rates on tomato were similar to survival rates on guava.

These results indicated that adults of the Asian citrus psyllid do not survive for very long when confined to guava, cotton, or tomato in a no-choice situation, with relatively high rates of mortality occurring within 2 d and greater than 95% mortality occurring within 6 to 9 d. Psyllids observed on guava, cotton or tomato during the studies were often in what appeared to be a feeding position but, based on the poor survival of adults, the psyllids were either unable to obtain from these plants nutrients required for survival, or chemicals associated with the plants were toxic. There was little evidence from these no-choice feeding experiments in the greenhouse that any of the guava cultivars might be much different than cotton or tomato with respect to their effect on survival of adult psyllids in a grove.

### Adult settling behavior and survival in choice situations

During the first 8 h after releasing adults into the cages in experiment 1, the adults in one cage containing only citrus (replication 7) congregated in the northeast end of the cage and remained there. By the next morning, 39 of the 40 adults were found dead on the floor of this cage in the northeast corner. A similar incidence occurred but at a lower death rate in two other cages containing only citrus, with 15 and 16 dead adults found on the northeast floor of each cage. The attraction of psyllids to the northeast end of the cages was attributed to their strong attraction to sunlight and the particular locations of the three cages in the greenhouse. Replication seven was dropped from the analyses. Mean  $\pm$  SEM air temperature in the greenhouse during the experiment was  $24.4 \pm 0.1$  °C ( $75.9 \pm 0.2$  °F).

There was no significant difference in the first experiment between the two treatments (citrus alone or citrus plus guava)

with respect to numbers of adult ACP settled on citrus plants during the first 3 h after adults were released into the cages (Fig. 2a). Significantly fewer adults were observed on citrus in cages containing both citrus and guava after 5, 7, and 8 h during the first day of the experiment and on 12 of the following 13 daily observation dates. Mean numbers of eggs laid per flush shoot were significantly lower on citrus in cages containing both citrus and guava on one of three sample dates (Table 1). However, conclusions from this experiment regarding the effect of guava on numbers of eggs laid on citrus were confounded by a limited

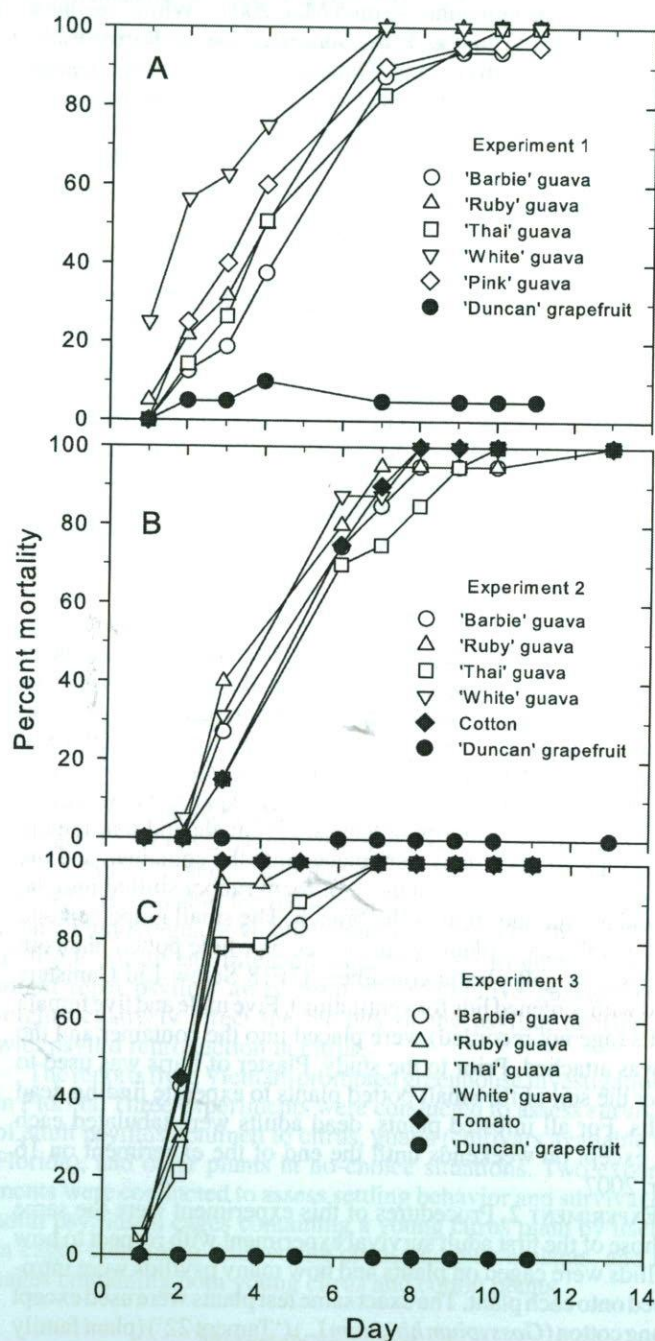


Fig. 1. Survival of adult *Diaphorina citri* caged in no-choice situations. (A) citrus ('Duncan' grapefruit) compared to five guava cultivars ('Barbie', 'Pink', 'Ruby', 'Thai', and 'White'); (B) same as A except cotton substituted for 'Pink' guava; (C) same as A except tomato substituted for 'Pink' guava.



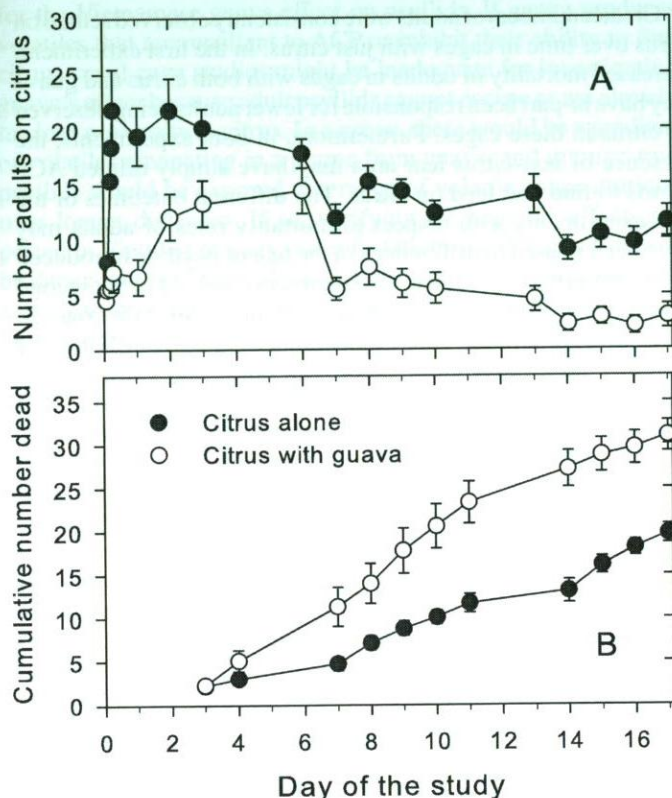


Fig. 2. Settling behavior and survival of *Diaphorina citri* in cages with citrus alone or with both citrus and 'White' guava (starting population of 40 psyllids per cage): (A) mean number and SEM of adults settled on citrus; (B) cumulative mean number and SEM of dead adults.

amount of flush suitable for oviposition for multiple females in each cage. Significant linear relationships were found between cumulative mean number of dead adults per cage (Y) and day of the study (X) for both citrus alone and citrus plus guava (Fig. 2b). For adults in cages with citrus alone:  $Y = 3.4 + 1.1X$ , slope SEM = 0.1,  $F = 149.9$ ,  $df = 125$ ,  $Pr > F < 0.0001$ ,  $r^2 = 0.55$ . For adults in cages with both citrus and guava,  $Y = 2.4 + 1.8X$ , slope SEM = 0.1,  $F = 265.8$ ,  $df = 125$ ,  $Pr > F < 0.0001$ ,  $r^2 = 0.68$ . The slopes from these regressions were significantly different, indicating that mortality rates were faster among adults in cages with both citrus and guava. A  $t$ -test indicated that the mean number of dead psyllids found each day differed significantly between cages with citrus (mean  $\pm$  SEM of  $1.5 \pm 0.1$ ) versus cages with both citrus and guava (mean  $\pm$  SEM of  $2.3 \pm 0.2$ ) ( $t = -2.85$ ,  $df = 178$ ,  $Pr > |t| = 0.0049$ ).

Mean  $\pm$  SEM air temperature in the greenhouse during the

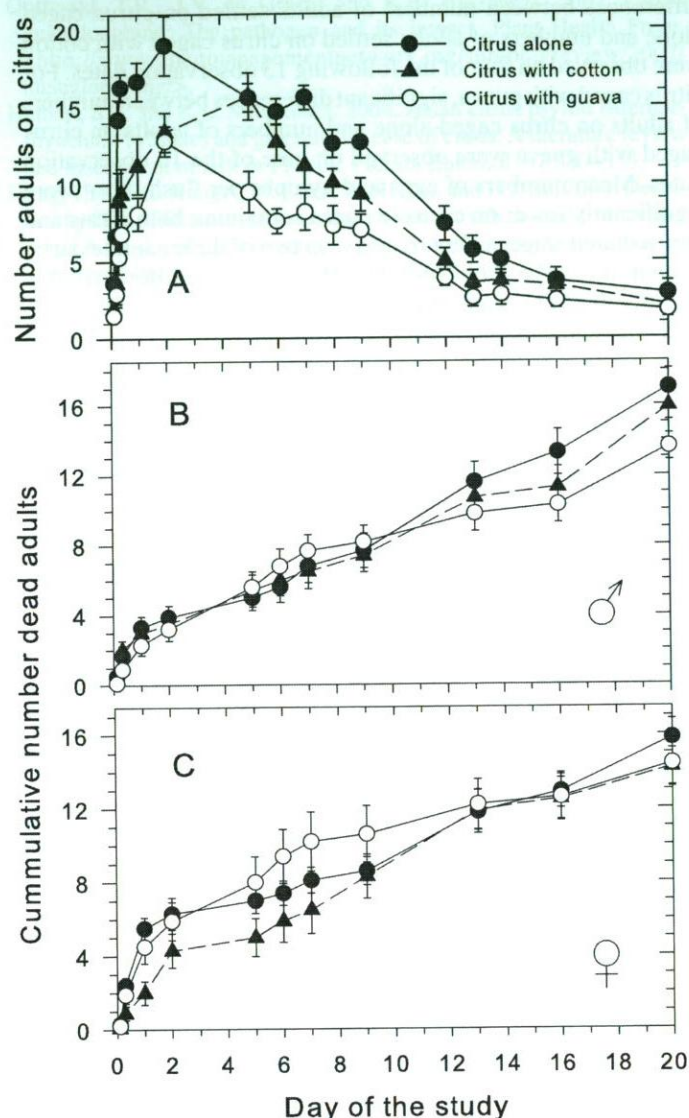


Fig. 3. Settling behavior and survival of *Diaphorina citri* in cages with citrus alone, citrus with cotton, and citrus with 'White' guava (starting population of 40 psyllids per cage): (A) mean number and SEM of adults settled on citrus; cumulative mean number and SEM of dead (B) males and (C) females.

second experiment was  $27.8 \pm 0.02^\circ\text{C}$  ( $82.0 \pm 0.1^\circ\text{F}$ ). Significantly greater numbers of adults were observed on citrus caged alone than on citrus caged with either guava or cotton during the first 2 d after psyllids were introduced into the cages (Fig. 3a). Significant

Table 1. Mean (SEM) number of *Diaphorina citri* eggs and nymphs/shoot on citrus in a cage with and without a guava plant (settling behavior and survival experiment 1). All infested flush shoots were removed on each observation day. Mean  $\pm$  SEM air temperature in the greenhouse during the experiment was  $24.4 \pm 0.1^\circ\text{C}$  ( $75.9 \pm 0.2^\circ\text{F}$ ).

Treatment	Mean (SEM) no. of immature <i>D. citri</i> per flush shoot			
	Day 7 <sup>z</sup>	Day 14 <sup>y</sup>	Day 18 <sup>x</sup>	Overall <sup>w</sup>
Citrus alone	318.7 (88.8)a	95.0 (10.2)a	48.5 (14.4)a	142.8 (34.8)a
Citrus with guava	275.9 (104.5)a	49.0 (14.5)b	33.2 (12.5)a	115.9 (39.5)a

<sup>z</sup> $t = 0.31$ ,  $df = 13$ ,  $Pr > |t| = 0.76$ .

<sup>y</sup> $t = 2.60$ ,  $df = 14$ ,  $Pr > |t| = 0.02$ .

<sup>x</sup> $t = 0.80$ ,  $df = 16$ ,  $Pr > |t| = 0.43$ .

<sup>w</sup> $t = 0.51$ ,  $df = 47$ ,  $Pr > |t| = 0.61$ .



differences between numbers of adults settled on citrus caged alone and numbers of adults settled on citrus caged with cotton were observed on four of the following 13 observation dates. For citrus caged with guava, significant differences between numbers of adults on citrus caged alone and numbers of adults on citrus caged with guava were observed on nine of the 13 observation dates. Mean numbers of eggs and nymphs per flush shoot were significantly lower on citrus in cages containing both citrus and guava than in cages with just citrus on two of three sample dates, though cumulative numbers were not significantly different (Table 2). Cumulative mean numbers of dead adults observed during the study indicated that mortality rates of male (Fig. 3b) and female (Fig. 3c) ACP were similar among cages with citrus alone, citrus with guava, and citrus with cotton. Regression analyses indicated similar mortality rates of adults in cages with citrus alone, citrus with guava, and citrus with cotton (Table 3). Means  $\pm$  SEM of  $3.1 \pm 0.3$ ,  $2.8 \pm 0.3$  and  $2.6 \pm 0.2$  dead psyllids were found each observation day in cages with citrus, citrus and cotton, and citrus and guava, respectively. There were no significant differences among these means ( $F = 1.6$ ,  $df = 319$ ,  $Pr > F = 0.03$ ; main effect  $F = 0.7$ ,  $df = 2$ ,  $Pr > F = 0.49$ ).

Adult Asian citrus psyllids introduced into cages generally moved to citrus faster when citrus was alone than when citrus was with guava. The increase in overall plant surface area in cages with guava might have been involved since psyllids were also slow to infest citrus in cages with cotton. Unfortunately, little is known regarding how ACP finds a host plant. There may be volatiles associated with citrus that attract migrating psyllids from long distances, or there may be citrus volatiles that attract psyllids that are already in close proximity. In addition to possible plant volatiles that attract the psyllid, there may be visual cues that attract adults to plants from short or long distances. Such visual cues might be missing in cage studies.

Greater numbers of adults were consistently observed settled on citrus over time in cages with just citrus. In the first experiment, increased mortality of adults in cages with both citrus and guava may have in-part been responsible for fewer adults being observed on citrus in these cages. Furthermore, in both experiments, the presence of non-citrus leaf area may have simply diluted ACP efforts to find and feed on citrus. The different outcomes of the two experiments with respect to mortality rates of adults may have been related to differences in the age of psyllids introduced into cages (16 d old in the first experiment, 10 d old in the second experiment) or differences in air temperatures. Mortality rates in cages with citrus alone were similar in each experiment, but lower mortality rates occurred in cages with citrus and guava during the second experiment (hotter conditions) than during the first experiment (cooler conditions). In contrast, adult ACP survival is usually longer under cooler conditions (Liu and Tsai 2000).

Reports from Vietnam indicate interplanting guava with citrus reduces infestations of ACP and its spread of citrus greening disease in citrus. In our greenhouse studies when citrus and guava were caged together, significant reductions sometimes occurred in numbers of adults settled on citrus. Numbers of eggs laid on citrus were sometimes reduced and adult survival was reduced in one experiment. We considered these reductions notable but less than anticipated. It is also notable that the presence of cotton with citrus did not produce markedly different psyllid responses than the presence of guava with citrus. It is possible that guava influence on psyllid behavior may have been more pronounced if a citrus genotype other than grapefruit had been studied. The 'White' guava plants used in our settling behavior and survival studies were genetically similar but not identical to the 'Bom' and 'Xaly' white guavas interplanted with citrus in Vietnam (Stover et al., 2008). It was therefore possible that, due to genetic differences, our guava plants were deficient in the traits responsible

Table 2. Mean number of immature (eggs and nymphs) *Diaphorina citri* on citrus in cages with citrus alone, citrus and cotton, or citrus and 'White' guava (settling behavior and survival experiment 2). All infested flush shoots were removed on each observation day. Mean  $\pm$  SEM air temperature in the greenhouse during the second experiment was  $27.8 \pm .02$  °C ( $82.0 \pm 0.1$  °F).

Treatment	Mean (SEM) no. of immature <i>D. citri</i> per flush shoot <sup>a</sup>			
	Day 7	Day 10	Day 20	Overall
Citrus alone	252.5 (31.8)a	112.8 (11.2)a	24.9 (3.7)a	93.5 (10.8)a
Citrus with cotton	235.9 (28.3)a	119.8 (17.2)a	11.6 (1.7)b	85.1 (10.7)a
Citrus with guava	152.7 (19.3)b	91.4 (12.5)a	16.7 (3.6)b	71.5 (8.0)a

<sup>a</sup>For each column, means followed by the same letter are not significantly different ( $\alpha = 0.05$ ), Ryan-Einot-Gabriel-Welsch test.

Table 3. Mortality rates of male and female *Diaphorina citri* in cages containing citrus, citrus and cotton, or citrus and guava based on simple linear regression of cumulative mean number of dead adults (Y) on day of the study (X) (settling behavior and survival experiment 2).<sup>a</sup>

Sex	Treatment	Regression	Slope SEM	r <sup>2</sup>	F
Male	Citrus alone	$Y = 1.5 + 0.8X$	0.04	0.76	342.1
	Citrus with cotton	$Y = 1.7 + 0.7X$	0.04	0.73	287.6
	Citrus with guava	$Y = 1.8 + 0.6X$	0.04	0.70	253.8
Female	Citrus alone	$Y = 3.2 + 0.6X$	0.04	0.71	263.2
	Citrus with cotton	$Y = 1.5 + 0.7X$	0.05	0.68	230.3
	Citrus with guava	$Y = 3.7 + 0.6X$	0.06	0.49	104.6
Over both	Citrus alone	$Y = 4.9 + 1.4X$	0.06	0.82	507.1
	Citrus with cotton	$Y = 3.1 + 1.4X$	0.06	0.82	478.8
	Citrus with guava	$Y = 5.7 + 1.3X$	0.09	0.65	202.4

<sup>a</sup>For each regression,  $df = 109$  and  $Pr > F = <0.0001$ .



for the Vietnamese guava effect on psyllids. If guava produces volatiles that are repellent to ACP or inhibit their ability to find citrus, small cage studies might be inadequate for investigating guava's effect because adult psyllids cannot escape or are already in close proximity to citrus. In a grove, there would be a gradient of volatiles emanating in a plume from guava, and immigrating psyllids would be exposed to a range of volatile concentrations over longer distances. If so, verifying the negative effects reported in Vietnam of guava on infestations of ACP in citrus will be dependent on field studies. Clearly, any guava effect on ACP and ultimately citrus greening disease is not simple and many factors need consideration in further research.

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